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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jiangchun Xu
Application No.:	09/347,496
Filed:	July 2, 1999
For:	COMPOUNDS FOR IMMUNOTHERAPY AND DIAGNOSIS OF COLON CANCER AND METHODS FOR THEIR USE

Examiner	:	Jehanne Souaya
Art Unit	:	1634
Docket No.	:	210121.471C1
Date	:	August 26, 2002

DECLARATION OF GARY FANGER, PH.D. UNDER 37 C.F.R. § 1.132

Commissioner of Patents
Washington D.C. 20231

The undersigned, Gary Fanger, Ph.D., hereby declares:

1. I am a Scientist at Corixa Corporation, the assignee of the subject application. The following analysis was performed by me.

2. I have reviewed the Office Action dated March 27, 2002 and in particular the rejection under C.F.R. § 112, first paragraph, and am familiar with the instant application. I provide this Declaration to assist the Examiner in analyzing the specification.

3. Tissue expression of the colon-specific protein, C888P (L1-cadherin; protein sequence encoded by GenBank Accession Number NM_004063, the full-length polynucleotide sequence comprising SEQ ID NO:21 of the above-identified application), was analyzed by immunohistochemistry (IHC) as follows:

To determine the tissue expression pattern of C888P, immunohistochemistry (IHC) analysis was performed on a normal colon and colon cancer tissue sections using purified polyclonal antibodies generated against purified Ra12-C888P protein. Tissue samples were fixed in formalin solution for 12-24 hrs and embedded in paraffin before being sliced into 8 micron sections. Steam heat induced epitope retrieval (SHIER) in 0.1 M sodium citrate buffer (pH 6.0) was used for optimal staining conditions. Sections were incubated with 10% serum/PBS for 5 minutes. Primary antibody was added to each section for 25 minutes followed by 25 minute incubation with anti-rabbit biotinylated antibody. Endogenous peroxidase activity was blocked by three 1.5 minute incubations with hydrogen peroxidase. The avidin biotin complex/horse radish peroxidase (ABC/HRP) system was used along with DAB chromogen to visualize antigen expression. Slides were counterstained with hematoxylin to visualize cell nuclei.

IHC analysis indicated that the antibodies generated against C888P are immunoreactive with colon cancer tissue. IHC staining indicated that expression of C888P is plasma membrane associated in both colon cancer and normal colon tissue. However, staining patterns between normal colon and colon tumor tissue are distinct. In normal colon tissue, expression of C888P is localized to the epithelial cell population. However, in colon cancer tissue, the anti-C888P staining indicated a uniform expression across the cancerous tissue. Thus, these antibodies can be used in IHC to differentiate normal colon from colon cancer tissue.

C888P expression was further analyzed in a variety of tissues including colon cancer sections and normal tissues. This analysis confirmed C888P positive membrane staining in 7 of 7 colon cancer samples. Positive membrane staining was also observed in normal colon, sigmoid colon, duodenum, ileum, appendix, and gallbladder. Marginal staining was seen in normal salivary gland and stomach. The following normal tissues were all negative for C888P staining: nasal mucosa, liver, heart, lung, esophagus, pancreas, antrum of stomach, seminal vesicle, testis, epidydimis, thyroid, breast, endometrium-proliferation, endometrium-secretory, myometrium, kidney, adrenal medulla, spleen, skeletal muscle, brain-white and gray matter, and spinal cord.

Thus, this IHC analysis confirms the expression of C888P in colon cancer and normal colon tissues as compared to a variety of other normal tissues and further validates this antigen as a marker for colon cancer.

4. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Gary Fanger, Ph.D. Date 8/22/02

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DECLARATION OF SUSAN HARLOCKER, PH.D. UNDER 37 C.F.R. § 1.132

Commissioner of Patents
Washington D.C. 20231

The undersigned, Susan Harlocker, Ph.D., hereby declares:

1. I am a Scientist at Corixa Corporation, the assignee of the subject application. The following analysis was performed by me.

2. I have reviewed the Office Action dated March 27, 2002 and in particular the rejection under C.F.R. § 112, first paragraph, and am familiar with the instant application. I provide this Declaration to assist the Examiner in analyzing the specification.

3. A nucleotide sequence alignment of SEQ ID NO:21 and L1-Cadherin (GenBank Accession Number NM_004063), was made and is shown in Figure

1. These sequences have structural similarity as is evidenced by the alignment of SEQ ID NO:21 over the entirety of its length to the sequence of L1-Cadherin from nucleotides 1666-2011. As is shown in Figure 1, with the exception of a single n at nucleotide 1755 and an additional a at nucleotide 2009 near the end of SEQ ID NO:21, the 2 sequences are 100% identical over the length of SEQ ID NO:21. Given the location and nature of these differences, all of these differences can be attributed to sequencing errors. Therefore, SEQ ID NO:21 is a fragment of L1-Cadherin.

4. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Susan L Harlocker
Susan Harlocker, Ph.D.

8/22/02
Date